ORIGINAL ARTICLE

Antimicrobial Susceptibility Pattern of Isolates From Diabetic Foot Ulcers

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ABSTRACT

Objective: To identify different organisms from diabetic foot ulcers and their antibiotic susceptibility.

Study Design: It was an institution based descriptive cross sectional study.

Place and Duration of Study: The study was conducted at Rehman Medical Institute, Hayatabad, Peshawar from 1st June 2017 to 31st December 2018.

Materials and Methods: A total of 88 specimen have been collected from a sample of 60 diabetic patients who have clinically infected foot ulcers and 99 pathogens wereisolated. The samples includes pus, tissue and fluid under study were cultured on blood agar and McConkey plates. Anaerobic culture medium 1 (AN1) was used for isolating anaerobes. The micro-organisms were identified through gram staining, culture and analytical profile index 20E. The sensitivity to a particular antibiotic was determined by Kirby-Bauer disk diffusion method.Anti-microbial susceptibility testing of isolates was performed as per the guidelines recommended by the Clinical Laboratory Standard Institute (CLSI).

Results: The most commonly isolated organisms were Escherichia coli (36.2%), Staphylococcus aureus (80%), Klebsiella pneumoniae (13%) and Pseudomonas aeruginosa (11.6%). Polymicrobial growth was found in 19 cultures. The ratio of gram-negative and gram-positive organisms isolated was 2.3. Gram-negative bacteria accounted for 69.7%, while gram-positive bacteria accounted for 30.3%. Staphylococcus aureus (87%) isolates were resistant to penicillin, ampicillin, levofloxacin, ceftriaxone & cefoxitin. High levels of resistance to amoxicillin/Clavulanic acid 15 (93.57%), ampicillin 20 (100%), ciprofloxacin 17 (89.47%) and co-trimoxazole 12 (85.71%) was seen in Escherichia coli.

Conclusion: E. coli are the most common pathogen isolated from diabetic foot ulcers. Their antibiograms suggest that resistance is on the rise and antimicrobial therapy should be selected based on culture results and antimicrobial sensitivity patterns.

Key Words: *Anti-Bacterial Agents, Chronic Disease, Diabetic Foot, Gram Negative Bateria, Gram Positive Bacteria, Staphylococcus Auerus.*

Introduction

Diabetes mellitus is a chronic disease which affects a large portion of our population. The history of diabetic foot ulcers is a serious concern as the risk of expiry at five years of a patient is 2.5 times more as compared to patients with diabeties not having a foot ulcer.[2] A recent report shows that the prevalence of diabetes mellitus is 11.77% in Pakistan and is expected to rise.[1] It is a health concern associated with major complications such as foot ulcers, retinopathy, nephropathy, and neuropathy.[2,3] The incidence of foot ulcers in diabetics ranges from 6% to 11% according to several reports.[4,5] The estimated lifetime risk of developing a foot ulcer is 15% - 25%.[6] A major concern for diabetic patients is foot ulcers as they frequently lead to amputation of the lower extremity and are a source of morbidity. In a study, almost 21.5% of patients with diabetic foot infection underwent minor or major amputationat some point intheir treatment or life.[7]

Diabetic foot infections are either monomicrobial or polymicrobial and the most common infecting organisms are Staphylococcus aureus, *Escherichia coli*, Pseudomonas aeruginosa, and Enterobacter.[8,9] Early recognition of the lesion and immediate initiation of appropriate antimicrobial therapy based on culture and antimicrobial susceptibility tests is crucial for controlling the infection, improving quality of life and preventing morbidity. One of the

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challenges in managing microbial infections is the emergence of multi-drug resistance mechanisms in some microbes such as Pseudomonas aeruginosa and Staphylococcus aureus rendering them less susceptible to antimicrobial agents.[10] This limits our choices of effective antibiotics making the treatment of diabetic foot more complicated and difficult. Antimicrobial susceptibility test is a prerequisite for treating diabetic foot infections which can help us choose effective therapeutic regimens.

The rationale behind the study is that antimicrobial susceptibility tests should be done frequently considering the emergence and rise of multi-drug resistant (MDR) organisms so the changing trends in the susceptibility patterns of the microbes are known. The objective of this study is to identify different organisms from diabetic foot ulcers and their antibiotic susceptibility.

Materials and Methods

This was an institution based descriptive cross sectional study conducted at Rehman Medical Institute. A sample of 60 diabetic patients with clinically infected foot ulcers admitted in RMI (Rehman Medical Institute), over the period from 1st June 2017 to 31st December 2018, they were identified through request forms sent to the microbiological laboratory of the institute. The forms consist of patients demographic and clinical details and are signed by the treating consultant. The study started after obtaining clearance from the institute's ethics review board.

Patients with diabetic foot infections are being included in this study if they have had an infected ulcer or wound or previous amputation. Exclusion criterion is non-diabetic patient open wound infections.

Age, gender, nature of clinical specimen, species of isolated pathogen and antibiogram of pathogens are recorded from the hospitals clinical microbiological laboratory.

Specimens are obtained after the wound had been washed vigorously with saline and debrided. The base of the ulcer is scraped with sterile curette to obtain specimen and sent to the lab for culture and antibiotic sensitivity testing. The samples which includes pus, tissue and fluid are processed for isolation of aerobes and anaerobes. The samples which includes pus, tissue and fluid are cultured on blood agar and McConkey plates. Anaerobic culture medium 1 (AN1) is used for isolating anaerobes at an incubation temperature of 37°C. The micro-organisms are identified through gram staining, culture and analytical profile index 20E. The sensitivity/resistance to a particular antibiotic is determined by Kirby-Bauer disk diffusion method and extended spectrum ß lactamase producers are identified by ß lactamase inhibitor combination.

Isolates are tested for antimicrobial susceptibility by the standard disk diffusion method following the guidelines recommended by the Clinical Laboratory Standards Institute (CLSI). 11 Disk diffusion method on Mueller-Hinton agar plates is used to test for antibiotic sensitivity. The disks are dried and stored in the refrigerator. After drying the plate, antibiotic discs (6 per 9cm plate) are applied. The isolate is scored resistant or susceptible based on CLSI guidelines 11. After parametric data collection, the data is analyzed and compiled in tabular form in Microsoft Excel 2016. The statictical test applied for calculation of *p* value is t-test which yielded a p-value of less than 0.05 which is regarded as statistically significant.

Results

A total of 60 patients were analyzed for this study, out of which 16 (26.6%) were females and 44 (73.3%) were males. Their age ranged between 21 and 86 years and the mean age of subjects was 52.88±14.7 specimens were collected from 60 patients and 99 pathogens were isolated.

Table I: Profile of Bacteria Isolated from Infected Foot Ulcers in Diabetic Patients Specimens (99 Isolates)

Bacteria category	Frequency (%)
n Isolates	
Aerobic and facultative isolates	
Gram negative	(n=69)
E. coli	25 (36.2%)
Klebsiella pneumonia	9 (13%)
Pseudomonas aeruginosa	8 (11.6%)
Acinetobacter	7 (10.14%)
Pseudomonas species	2 (2.89%)
Proteus mirabilis	9 (13%)
Proteus vulgaris	2 (2.89%)
Klebsiellaoxytoca	2 (2.89%)
Morganellamorganii	2 (2.89%)
Citrobacter species	1 (1.44%)
Enterobacter species	2 (2.89%)
Gram positive	(n=30)
Staphylococcus	24 (80%)
Enterococcus species	6 (20%)

Polymicrobial growth was found in 19 cultures. The ratio of gram negative and gram-positive organisms isolated was 2.3. Gram-negative bacteria accounted for 69 (69.7%), while gram-positive bacteria accounted for 30 (30.3%). Organism isolated from different cultures are summarized in Table I.

Table II: Antimicrobial Proportion Resistance (%) and It's Pattern of Gram-Positive Bacterial Isolates from Infected Foot Ulcers in Diabetic Patients

Proportion Resistance (%)		
Antimicrobial agent	**Staphylococcus (n=24)**	**Enterococcus (n=6)**
Amikacin	3(17.65%)	
Amoxicillin	1 (100.00%)	
Amoxicillin/Clavulanic acid	2 (100.00%)	0 (0%)
Ampicillin	4 (100.00%)	2 (100%)
Ampicillin/Sulbactam	1 (100.00%)	
Cefaclor	2 (100.00%)	
Cefazolin	2 (100.00%)	
Cefoxitin	20 (86.96%)	
Cefpodoxime	2 (100.00%)	
Ceftizoxime	3 (100.00%)	
Ceftriaxone	3 (100.00%)	
Cefuroxime	3 (100.00%)	
Cephalexin	3 (100.00%)	
Chloramphenicol	4 (16.67%)	3 (60%)
Ciprofloxacin	12 (92.31%)	4 (100%)
Clarithromycin	0 (0.00%)	
Clindamycin	5 (21.74%)	
Co-trimoxazole	2 (18.18%)	
Doxycycline	3 (12.50%)	4 (100%)
Erythromycin	18 (81.82%)	5 (100%)
Gentamicin	10 (52.63%)	5 (83.33%)
Levofloxacin	15 (100.00%)	
Linezolid	1 (4.35%)	0 (0%)
Minocycline	6 (27.27%)	5 (83.33%)
Moxifloxacin	1 (25.00%)	
Norfloxacin		
Penicillin	20 (100.00%)	2 (50%)
Rifampicin	3 (13.64%)	5 (100%)
Teicoplanin		1 (25%)
Tigecycline		0 (0%)
Vancomycin	1 (4.35%)	2 (33.33%)

The results of susceptibility tests are summarized in Table II and III. Table II shows the antimicrobial susceptibility patterns of gram-positive isolates from specimens. Almost all of Staphylococcus aureus (87%) isolates were resistant to penicillin, ampicillin, levofloxacin, ceftriaxone & Cefoxitin. Approximately 18 (81%) exhibited resistance to erythromycin whereas 1 (4.3%) was resistant to vancomycin.

Table III shows the antimicrobial susceptibility patterns of gram negative isolates from specimens. High levels of resistance to amoxicillin/Clavulanic acid 15 (93.57%), ampicillin 20 (100%), ciprofloxacin 17 (89.47%) and co-trimoxazole12 (85.71%) was seen in *Escherichia coli*. More than half of the isolates of Pseudomonas aeruginosa were resistant to ciprofloxacin, gentamicin, imipenem, and levofloxacin.

Discussion

Diabetic foot ulcers are often non-healing due to several underlying factors and complications such as neuropathy, peripheral vascular disease and high plantar pressures[12], they are one of the main reasons of hospitalization of persons with diabetes with super added infections and subsequent impaired healing. Proper management and care of diabetic foot infection can decrease the frequency of infection-associated morbidity, need for hospitalization and incidence of limb amputations. One of the reasons for poorly managed diabetic infections are the lack of understanding of the microbial prevalence and effective therapeutic regimens.[13]

In this study, the predominant pathogens are gram-negative bacteria; *Escherichia coli* being common etiological agent followed by *Staphylococcus aureus* and *Klebsiella pneumonia*. Similarly, in another study gram-negative bacteria were also the most common pathogen with *Proteus Mirabilis* being the dominant one.[14] There seems to be a change in the trend of the organisms causing infections as earlier studies have shown gram-positive bacteria as the predominant organism causing diabetic foot infection.[15,16]

Polymicrobial infection are seen in 25% specimens, whereas in two other studies it was seen in 51% and 50% specimens.[14,17] Monomicrobial cultures are more than polymicrobial cultures (73 vs 15) in this study with an average of 1.12 pathogen isolated per specimen. This ratio is less, compared to other studies, whose ratios were 1.85 and 1.5.[14,18] This can be attributed to the less severity of the foot infection and low pathogenicity of isolated organisms in this study. Severe infections usually yield polymicrobial isolates and in some cases, three or more organisms may be cultured.[18] In our study, no culture yielded more than two pathogens. Mild infections are

Table III : Antimicrobial Proportion Resistance (%) and It's Pattern of Gram Negative Bacterial Isolates from Infected Foot Ulcers in Diabetic Patients (99 Isolates)

Antimicrobial Agent	Escherichia Coli	Klebsiella Pneumonia	Pseudomonas Aeruginosa	Acinetobacter	Pseudomonas A Species	Proteus Mirabilis	Proteus Vulgaris	Klebsiella Oxytoca	Morganella Morganii	Citrobacter Species	Enterobacter Species
N	25	9	8	7	2	9	2	2	2	1	2
Amikacin	3 (12%)	4 (44.44%)	4 (50%)	6 (100%)	1 (50%)	1 (11.11%)	1 (50%)	1 (50%)	0 (0%)	0 (0%)	0 (0%)
Amoxicillin	1 (100%)					1 (100%)					
Amoxicillin/ClavulanicAcid	15 (93.75%)	3 (75%)				5 (71.43%)	1 (50%)		2 (100%)		1 (100%)
Ampicillin	20 (100%)	8 (100%)	1 (100%)	4 (100%)		6 (75%)	1 (100%)	2 (100%)	2 (100%)	1 (100%)	1 (100%)
Ampicillin/Sulbactam	11 (91.67%)			1 (100%0		0 (0%)	0 (0%)				
Aztreonam	22 (88%)	8 (88.89%)	5 (71.43%)		1 (50%)	2 (22.22%)	0 (0%)	1 (50%)	0 (0%)	1 (100%)	
Cefazolin	1 (100%)										
Cefepime	20 (83.33%)	9 (100%)	5 (62.50%)	6 (100%)	1 (50%)	2 (33.33%)	1 (50%)	1 (50%)	1 (50%)	1 (100%)	
Cefixime	1 (100%)						1 (100%)				
Cefoperazone /Sulbactam	7 (38.89%)	3 (33.33%)	1 (100%)	6 (100%)		1 (12.5%)	0 (0%)	1 (50%)	0 (0%)	0 (0%)	0 (0%)
Cefotaxime	8 (80%)	2 (100%)		2 (100%)		0 (0%)	1 (100%)	1 (100%)	0 (0%)		1 (100%)
Ceftazidime	13 (81.25%)	5 (100%)	4 (50%)	7 (100%)	1 (100%)	1 (25%)	0 (0%)	1 (100%)	0 (0%)	1 (100%)	1 (100%)
Ceftriaxone	15 (83.33%)	8 (100%)		6 (100%)		2 (28.57%)	0 (0%)	1 (50%)	1 (100%)	1 (100%)	1 (100%)
Cefuroxime	5 (100%)										
Chloramphenicol						1 (100%)					
Ciprofloxacin	17 (89.47%)	5 (71.43%)	5 (62.50%)	6 (100%)	2 (100%)	3 (33.33%)	2 (100%)	0 (0%)	1 (50%)	0 (0%)	
Colistin/Polymixin B	0 (0%)	0 (0%)		0 (0%)		3 (60%)		0 (0%)	0 (0%)	0 (0%)	
Co-Trimoxazole	12 (85.71%)	4 (80%)		4 (100%)		6 (75%)	1 (100%)	1 (50%)	1 (100%)	1 (100%)	1 (100%)
Doxycycline	10 (76.92%)	3 (75%)		0 (0%)		1 (100%)	1 (100%)				
Erythromycin											
Gentamicin	10 (50%)	4 (57.14%)	4 (57.14%)	6 (100%)	1 (100%)	2 (40%)	1 (100%)	1 (50%)	1 (100%)	0 (0%)	
Imipenem	4 (20%)	3 (50%)	4 (57.14%)	3 (100%0	2 (100%)	3 (33.33%)	2 (100%	1 (50%)	1 (100%)	0 (0%)	
Levofloxacin	14 (77.78%)	3 (50%)	4 (57.14%)		1 (100%)	1 (14.29%)	1 (100%)	1 (50%)	0 (0%)		0 (0%)
Meropenem	2 (18.18%)	3 (42.86%)	1 (50%)	5 (100%)		1 (20%)		1 (50%)	0 (0%)		0 (0%)
Minocycline	3 (37.50%)			0 (0%)							
Piperacillin/Tazobactam	10 (41.67%)	4 (44.44%)	4 (57.14%)	7 (100%)	0 (0%)	1 (11.11%)	1 (50%)	1 (50%)	0 (0%)	0 (0%)	1 (50%)
Tobramycin	1 (100%)										

frequently monomicrobial.

In our study, Staphylococcus aureus isolates are found to be susceptible to vancomycin (95%) but in other studies, susceptibility to vancomycin was 100%.[14,17] Resistance is seen against most of the antibiotics such as penicillin, ciprofloxacin, ceftriaxone except gentamicin. A similar trend was seen in another study but in our case, erythromycin is resistant to most of the pathogens (81.82%) but in the referenced study, erythromycin was almost 70% sensitive.[14] Enterococcus showed varying susceptibility to antibiotics, but it is uniformly resistant to ampicillin and 33% resistant to vancomycin this is alarming because these results are different from results of a 2012 study done in Iran in which only 4% of *Enterococcus* isolates were resistant to ampicillin and almost all isolates were susceptible to vancomycin.[22] This shows that over the years the susceptibility pattern of *Enterococcus* is changing and empirical use of these antibiotics in diabetic foot ulcer (infected) should not be encouraged.

Of the 8 strains of Pseudomonas aeruginosa screened, more than half showed resistance to amikacin, gentamicin, and imipenem. Intermediate resistance is seen towards ciprofloxacin (62.5%). The resistance pattern of Pseudomonas aeruginosa is similar to findings of a study done in Italy in which the microbe showed similar resistance to ciprofloxacin but least resistance to amikacin which is a different pattern compared to our result.[19]

Proteus mirabilis strains are often least resistant to ciprofloxacin (33%) and gentamicin (40%) but resistance against ampicillin (75%) and amoxicillin/clavulanic acid (71.43%) was more. But, in a 2012 study done in Kuwait, Proteus mirabilis showed around 13% resistance towards ciprofloxacin, 23% towards gentamicin, 45% towards ampicillin and 28% towards amoxicillin/clavulanic acid. It is apparent that over the years, the resistance of antibiotics mostly used in empirical therapy is increasing and approach towards them should be reconsidered.[20]

Increased resistance to cefepime and ceftriaxone is observed among Escherichia coli and Klebsiellapneumoniae. Escherichia coliis least resistant to amikacin and meropenem. The resistance of many isolates of both species can be explained by the production of extended-spectrum beta-lactamases (ESBL) rendering them resistant to extended-spectrum cephalosporins.[21] Similar susceptibility results of E. coli were observed in a study conducted in India where resistance to cefepime was maximum and to meropenem was minimum.[23]

The limitations of our study included a lack of easy access for tissue cultures and clinical signs of infections. In future, more studies are recommended in this region to isolate the common organisms in diabetic foot patients and their susceptibility to different drugs at an earlier stage and to use appropriate antibiotics inorder to decrease Multi Drug Resistance (MDR) organisms.

Conclusion

This study shows that the most common pathogen isolated from diabetic foot ulcers is Escherichia coli. Multi-Drug-Resistant organisms are alarmingly high in the diabetic foot ulcers. Vancomycin, colistin/ polymixin, and imipenem are the only effective drugs against Multi-Drug Resistance organisms. The antibiogram of this study suggests that most pathogens remain sensitive to a few agents, but resistance is on the rise.

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